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(54) An agent for preventing plant virus diseases.

(57) An agent for preventing plant virus diseases contains polylysine as an active component. The polylysine used can be α-polylysine or, preferably, ε-polylysine.

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AN AGENT FOR PREVENTING PLANT VIRUS DISEASES

This invention relates to an agent for preventing plant virus diseases.

Usually, when a plant is infected with a virus disease, the whole plant is infected and dies readily. Also, the infected plant communicates the disease to other healthy plants one after the other, by contact or entomophilous contagion, and as a result a great deal of damage is done to the plants, before the plants are cropped or removed. Examples of viruses of these plant pathogens include the tobacco mosaic viruses (abbreviated as TMV), which infect the eggplant family, the potato viruses (abbreviated as PVX) and the cucumber mosaic viruses (abbreviated as CMV), which infect the eggplant, cucumber and rape families.

Methods which have been tried, for preventing these virus diseases include, for example, for entomophilous infection, such as the cucumber mosaic diseases caused by CMV, extermination of intermediate insects, change of seeding time to avoid the insect generating time, cultivation of specially bred plants and so on. However, these methods are insufficient to obtain a good control effect. For preventing the spread of virus diseases, infected plants have been pulled out. However, it is difficult to prevent poisonous insects flying in from other fields, so that this attempt at prevention is not effective.

On the other hand, TMV diseases, which do not infect by entomophily, but by contact contagion, infect crops at work by contact of the viruses with agricultural machines or by mixing the viruses into the soil. For preventing these diseases, agricultural machines, the soil, working clothes, fingers, etc. are subjected to disinfection at a high cost and involve expensive labour. However, such methods are insufficient to prevent these virus diseases from occurring.

Furthermore, TMV having a weakened poison effect has been tried recently for preventing TMV-tomato. However, there is no guarantee that this type of TMV acts effectively on other kinds of crops. On the contrary, there is the possibility that a tomato which has been contacted with the virus may form a contagion source and that viruses having a strengthened poison effect are mixed with viruses having the weakened poison effect, when many tomato seedlings are inoculated with the latter virus. For these reasons, no viruses having a weakened poison effect have come into wide use.

As described above, physical elimination or agronomic control are only considered as a counterplan for preventing plant virus diseases under existing circumstances. However, the control effect is small and extensive damage is done to crops by plant virus diseases. Accordingly, the prevention of plant virus diseases is an important subject for agriculture.

For research purposes into prevention agents for plant virus diseases, many antiviral activates prepared from many natural products or synthetic compounds have been investigated. As a result, some materials having antiviral activity have been found. Firstly, as a so-called multiplication control agent which controls virus multiplication in plant bodies, materials similar to nucleic acids such as 2-thiouracil, 8-azaguanine, 5-fluorouracil, etc. have been found. However, some of these materials generate mutants and inhibit protein metabolism in the hosts and give rise to damages. These materials are generally expensive and have little actual control effect in the field, so that these materials are not used in practice.

As an agent for blocking contact contagion, sodium alginate is used. However, its application is limited to the TMV-OM and the control effect is insufficient. The agent is ineffective with an entomophilous contagion virus such as CMV, which is carried by plant lice such as aphids.

As a result of an investigation into agents which reduce or eliminate the above problems and have good safety and preventive effects, the present inventors have found that polylysine has the effect of preventing plant virus diseases and have based the present invention upon this discovery.

The present invention provides an agent for preventing plant virus diseases, wherein the agent contains polylysine as an active component.

The polylysine used in the present invention is a polymer of lysine. Lysine is an essential amino acid having two amino groups in the molecule. Polylysine prepared from lysine comprises two types: α-polylysine, which is obtained by condensation of α-amino groups and carboxyl groups, and ε-polylysine, which is obtained by condensation of ε-amino groups and carboxyl groups.

When polylysine is synthesized by a chemical method, α-polylysine is usually obtained. The HBr salt of α-polylysine is commercially available as a reagent, e.g. from SIGMA Chemical Company, USA, and it can be used to carry out this invention. On the other hand, α-polylysine can be prepared by zymotechnics, for instance. In this method, ε-polylysine is obtained by incubation of a certain kind of micro-organism belonging to the Streptomyces genus under aerobic conditions (Japanese Patent Publication No. 59-20359). For instance, No. 346-D strain (Deposit No. 3834 of microorganisms of FRI), Streptomyces albulus subsp.

lysinopolymerus, which belongs to the Streptomyces genus and is a polylysine-producing actinomycetes, is incubated on culture medium. ε-Polylysine can be obtained by separating and collecting from the resulting cultures. Naturally, the methods for producing polylysine which are used in the present invention are not limited at all.

The purpose of the present invention is attainable both with α-polylysine and ε-polylysine. However, ε-polylysine is preferably used, as the agent for preventing plant virus diseases. The reason is that, although any polylysine can be used in accordance with the present invention, the effect of ε-polylysine is slightly better than that of α-polylysine for preventing the infection of virus diseases, as shown by the following data. Accordingly, when carrying out the present invention, a more desirable effect can be obtained by using ε-polylysine.

Table 1. Effect of polylysine for preventing infection

Polylysine Concentration	Rate of Prevention of Infection (TMV)	
	α-Polylysine hydrobromide	ε-Polylysine hydrochloride
200 ppm	90%	100%
100 ppm	65%	91%
50 ppm	47%	67%
25 ppm	33%	49%

In this test, liquids having various concentrations of α-polylysine hydrobromide and ε-polylysine hydrochloride were prepared and the same amount of TMV (2 µg/ml) was mixed into each liquid, then each mixture was inoculated into Samson NN tobacco by the Carborundum method and local lesions were counted. The concentrations of each polylysine are represented by the concentrations of free polylysine. As a control, an equivalent mixture of distilled water and virus was used.

The inhibition rates of infection were calculated by the following equation.

Infection inhibition rate =

$$\frac{C-T}{T} \times 100\%$$

C: the number of local lesions in a control area

T: the number of local lesions in a test area

As polylysine has a free amino acid in its constituent molecule, it can be used in the free form. Usually, it can be used as salts of inorganic or organic acids. In the case of a α-polylysine, it can be obtained as an HBr salt by suitable restriction of the materials used in the synthetic reaction, but the other salts can be used if necessary. ε-Polylysine is frequently obtained in the form of a hydrochloride or it can be obtained in the free form. Any form of a salt of an inorganic acid, such as the hydrochloride or sulphate, and any salt of an organic acid such as the propionate or palmitate, can be used in carrying out the present invention.

As regards the preventive effects of polylysine on the virus, the obstruction effect upon virus multiplication can be exemplified, in addition to the inhibition effect of infection as shown in Table 1. Namely, various initial concentrations of ε-polylysine hydrochloride were put into a petri dish (diameter 9 cm, liquid volume 20 ml), after TMV (2 µg/ml) and PVX (10 g/ml) were inoculated into a leaf the day before, discs of 2 cm diameter were punched with a cork borer from the leaf infected by the virus, and the discs were floated on the petri dish. The virus was incubated under natural light at room temperature for three

days, then the discs were thoroughly washed with water and ground in a mortar containing ten times the volume of 1% K_2HPO_4. The resultant liquid of TMV was inoculated into Samsun NN tobacco and the resultant liquid of PVX was inoculated into xanthine tobacco and then the local lesions were tested. The results are shown in Table 2. As a control, discs of the infected leaf were floated on distilled water.

Table 2. Obstruction effect upon virus multiplication

ε-Polylysine concentration*	TMV	PVX
200 ppm	46%	61%
100 ppm	44%	63%
50 ppm	6%	39%
25 ppm	0%	15%

* These concentrations are based of the free ε-polylysine concentration.

Thus, when the concentration of ε-polylysine is above 100 ppm, the obstruction effect shown is around 50%.

Methods for preventing plant virus diseases by using polylysine according to this invention are as follows.

(1) 10 to 500 ppm of aqueous solutions of polylysine and sprayed or applied on plants to be prevented.

(2) While working by polling the tops, nipping out buds, binding props etc., workers disinfect agricultural tools such as shears and their fingers using 100-500 ppm of aqueous polylysine solution, whenever they treat a few crops.

(3) Before sowing is carried out, seeds are dipped into a 100-500 ppm of polylysine solution for a definite time.

Especially, for contagious viruses, before agricultural work such as polling the tops, nipping out buds, binding props, inducing etc., it is effective to spray an aqueous polylysine solution on every desired plant.

As virus diseases which can be prevented by using the polylysine of the present invention as a preventing agent, in addition to the above TMV, PVX, etc., CMV (cucumber mosaic virus), WMV (pumpkin mosaic virus), TuMV (turnip mosaic virus) and CaMV (cauliflower mosaic virus) can be mentioned by way of example.

When the plant virus diseases caused by these viruses are controlled, the preventive effect can be enhanced by

(1) simultaneous use of a spreader for an agricultural medicament and the preventing agent of this invention, or

(2) simultaneous use of a preventing agent for plant lice, such as aphids, and the preventing agent of the present invention. The simultaneous use or preparation can exceed the simple use of the polylysine of this invention in its preventive effect.

When the polylysine of the present invention is used as a preventing agent for plant virus diseases, its main advantage is enhanced safety and the heightened preventive effect obtained. When ε-polylysine hydrochloride, which is used in the present invention, is administered orally to mice, the acute toxicity LD_{50} is above 5g/kg. Furthermore, the polylysine of this invention is normal in mutagenesis tests with micro-organisms, the primary test of skin stimulus with rabbits and in the primary test of eye mucosa stimulus.

The effects of the present invention are as follows.

As described above, the preventing agent for plant virus diseases of the present invention, in which polylysine is used, shows a better preventive effect than that of

Example 2.

200 mg of ε-polylysine and 100 mg of sodium lauryl sulphate were homogeneously made up to 1 litre with water (aqueous solution of 200 ppm of ε-polylysine).

The aqueous solution prepared as described above was sprayed on every leaf of a potted tobacco (xanth-NC breed) seedling at the period of 10 leaves. One hour after spraying, previously prepared PVX (5 µg/ml) was inoculated into the sap of leaves with carborundum in the usual way. Four days after the inoculation, local lesions were examined and the infection inhibition rates for PVX were calculated in accordance with the method of Example 1.

Five roots per group were used in a test and the test was repeated twice. The test results are shown in Table 4.

Table 4

Agent Used	Concentration (ppm)	Infection Inhibition Rate (%)	Medical Damage
ε-Polylysine aqueous soln.	200 *	99	Nothing
Non-treatment	-		Nothing

* Concentration based on ε-polylysine.

Example 3.

200 mg of ε-polylysine and 200 mg of xanthan gum were homogeneously made up to 1 litre with water (aqueous solution of 200 ppm of ε-polylysine). The above-mentioned ε-polylysine aqueous solution was sprayed at the rate of 15 ml per pot on every seed leaf of a potted cucumber seedling, five days after it germinated. One hour after spraying, CMV was inoculated into the sap of leaves with carborundum in the usual way. Fourteen days after the inoculation, the number of roots have mosaic-lesions which appeared on the cucumber seedling were examined and the conventional preventing agents, so that the virus damage of useful plants can be prevented. Efficiency can also be heightened while carrying out agricultural work. Thus, the agent of the present invention can produce practical results.

The following non-limitative examples illustrate this invention more specifically.

Example 1.

α-polylysine-hydrobromide aqueous solution, which was adjusted to a concentration of 200 ppm based on α-polylysine, was sprayed on every leaf of a potted tobacco seedling (Samsun NN breed) at the period of 12 leaves. Thirty minutes after spraying, previously prepared TMV (1 ug/ml) was inoculated into the sap of the leaves with carborundum in the usual way.

Four days after the inoculation, local lesions were examined and the infection inhibition rates for TMV were calculated in accordance with the method described in relation to Table 1.

As a control agent, 5000 ppm of sodium alginate in aqueous solution was used.

Five roots per group were used in a test and the test was repeated twice. The test results are shown in Table 3.

3. An agent as claimed in claim 1 or 2, wherein the amount of ε-polylysine present is equivalent to a rate of use of 10-10,000 ppm.

4. An agent as claimed in claim 1, 2 or 3, wherein the polylysine is present in the form of a salt of an organic acid or an inorganic acid.

5. A method of propogating plants subject to a plant virus disease, which comprises applying to the plants or to tools or the hands as a disinfectant or to seeds prior to sowing an agent comprising polylysine.

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EUROPEAN SEARCH REPORT

Application Number

EP 87 30 5464

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
X	CHEMICAL ABSTRACTS, vol. 86, no. 3, 17th January 1977, page 65, no. 12233s, Columbus, Ohio, US; E. TYIHAK et al.: "Antagonistic effect on TMV infectivity between poly-l-lysine and poly-l-arginine", & ACTA PHYTOPATHOL. ACAD. SCI. HUNG. 1976, 11(1-2), 11-16	1-5	A 01 N 37/46
X	BIOLOGICAL ABSTRACTS, vol. 73, no. 1, 1982, page 565, no. 5442, Biosciences Information Service, Philadelphia, US; S. KAJITA et al.: "Effects of high molecular weight polycation and polyanion in the mechanical inoculation of tobacco mosaic virus", & VIRUS (TOKYO) 31(1): 33-40, 1981	1-5	
A	CHEMICAL PATENTS INDEX, Basid Abstracts Journal, Section C, week X/33, 1976, no. 62633x, Derwent Publications, Ltd, london, GB; & JP-A-51 076 430 (KYOWA HAKKO KOGYO K.) 02-07-1976	1-5	

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

A 01 N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	29-05-1989	DECORTE D.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

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